Supporting Information

pH-Responsive Nano-Self-Assemblies of the Anticancer Drug 2-Hydroxyoleic Acid

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Critical Packing Parameter model

The critical packing parameter (CPP) model (**Eq. S1**) considers the molecular geometries of the participating amphiphilic compounds to describe self-assembled structures, and is expressed as following:[1]

$$CPP = \frac{v}{a_0\, l} \tag{S1}$$

where v is the effective hydrophobic chain volume, a_o is the effective headgroup area at the interface, and l is the length of the hydrophobic tail. The CPP model takes into account the relative contribution of the hydrophobic and hydrophilic moieties of the amphiphilic compound in predicting the type of the self-assembled structure: at CPP > 1, inverse (oil-continuous) structures will be formed, whereas at CPP ~ 1 and CPP < 1, the compound will tend to form flattened bilayers (a lamellar liquid crystalline structure), and normal (water-continuous structures), respectively. [1]

SAXS data analysis

The space groups of the corresponding LLC phases were determined by the relative positions of the detected Bragg peaks in the scattering curves, which correspond to the reflections from the planes defined by their Miller indices. The lattice parameters of the inverted-type hexagonal H_2 phase, a_{H_2}, and of the bicontinuous cubic phases *Pn3m*, a_{Pn3m}, and *Im3m*, a_{Im3m}, the interlamellar distance of the multilamellar vesicles' (MLVs') bilayer stacks, d, was calculated from the q positions of the Bragg reflections using **Eq. S2-S4**. Lorentzian fit was used to estimate the q- value of Bragg reflections.

$$a_{H_2} = \frac{4\pi}{q\sqrt{3}}\sqrt{h^2 + hk + k^2} \tag{S2}$$

$$a_{Pn3m/Im3m} = \frac{2\pi}{q}\sqrt{h^2 + k^2 + l^2} \quad \text{(S3)}$$

where q is the q-value and h, k, and l are the Miller indices of the corresponding Bragg reflections.

$$d = \frac{2\pi h}{q_h} \quad \text{(S4)}$$

where h is the order of the Bragg peak, and q_h is the q-value of the h-th order Bragg peak.

Fitting of $pK_a{}^{app}$ from the ζ –potential measurements

To estimate the apparent pK_a of 2OHOA in the 2OHOA/GMO self-assemblies, $pK_a{}^{app}$, the ζ –potential values in the pH 2.0-9.0 range, ζ_{pH} using **Eq. S5**, derived from the Henderson-Hasselbalch relation[2],

$$\zeta_{pH} = Z1 + \frac{Z1 - Z2}{10^{(pK_a^{app} - pH)*p} + 1} \quad \text{(S5)}$$

where the fitting parameters *Z1* and *Z2* are the bottom and top asymptotes, and p is the hill slope.

Table S1. Lattice parameters of the identified liquid crystalline phases in samples with 1/1 and 3/7 2OHOA/GMO mass ratio at different pH, calculated using **Eq. S2-S4**. Bonnet ratio is calculated as the ratio between the lattice parameters of the coexisting *Im3m* and *Pn3m* structures and has a theoretical value of 1.279.[3] All samples were dispersed in PBS with 2 mg/mL F127.

	2OHOA/GMO 1/1 mass ratio Lattice Parameters [nm]					**2OHOA/GMO 3/7 mass ratio** Lattice Parameters [nm]				
pH	a_{H2}	a_{Pn3m}	a_{Im3m}	*d*	*Bonnet ratio*	a_{H2}	a_{Pn3m}	a_{Im3m}	*d*	*Bonnet ratio*
2.0	5.8	8.0	-	-	-	-	8.4	-	-	-
3.0	5.9	8.3	11.1	-	**1.34**	-	8.5	-	-	-
3.5	-	9.1	12.0	-	**1.32**	-	9.3	12.3	-	**1.33**
4.0	-	9.9	12.9	-	**1.30**	-	-	14.6	-	-
4.5	-	12.3	15.3	-	**1.24**	-	-	-	vesicles	-
5.0	-	-	-	4.0	-	-	-	-	vesicles	-
6.0	-	-	-	4.0	-	-	-	-	vesicles	-
7.4	-	-	-	vesicles	-	-	-	-	vesicles	-

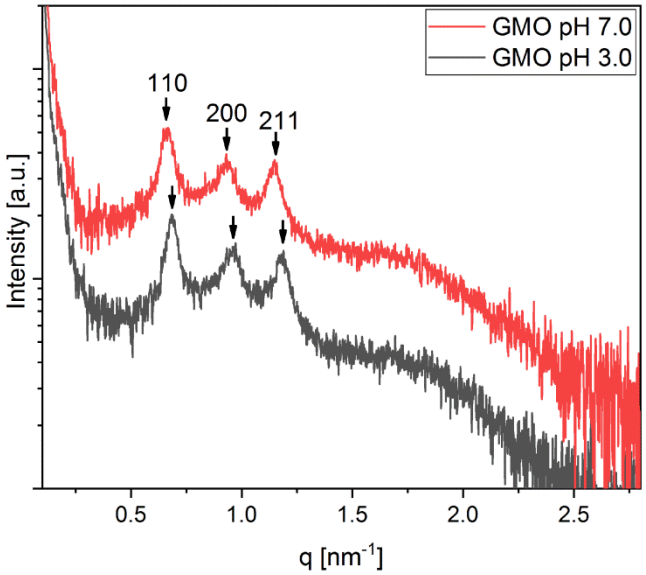

Figure S1. SAXS patterns for the F127-stabilized GMO cubosomes in absence of 2OHOA at pH 3.0 (black) and 7.0 (red), with the calculated *Im3m* phase lattice parameters of 13.1 and 13.5 nm, respectively. The change in pH from 7.0 to 3.0 resulted only in a very minor decrease in the lattice parameter.

References

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